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VPg unlinkase/TDP2 in cardiovirus infected cells: Re-localization and proteolytic cleavage

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ABSTRACT

Cardioviruses cause diseases in many animals including, in rare cases, humans. Although they share common features with all picornaviruses, cardioviruses have unique properties that distinguish them from other family members, including enteroviruses. One feature shared by all picornaviruses is the covalent attachment of VPg to the 5′ end of genomic RNA via a phosphotyrosyl linkage. For enteroviruses, this linkage is cleaved by a host cell protein, TDP2. Since TDP2 is divergently required during enterovirus infections, we determined if TDP2 is necessary during infection by the prototype cardiovirus, EMCV. We found that EMCV yields are reduced in the absence of TDP2. We observed a decrease in viral protein accumulation and viral RNA replication in the absence of TDP2. In contrast to enterovirus infections, we found that TDP2 is modified at peak times of EMCV infection. This finding suggests a unique mechanism for cardioviruses to regulate TDP2 activity during infection.

1. Introduction

The cardiovirus genus of the *Picornaviridae* family is divided into three species (A, B, C) and includes encephalomyocarditis virus (EMCV), Theiler's murine encephalomyelitis virus (TMEV), and the emerging human virus, Saffold virus (SAFV) (Ghildyal et al., 2009) [http://www.picornaviridae.com/cardiovirus/cardiovirus.htm]. EMCV is primarily known for diseases it causes in both wild and domestic animals worldwide, including mice, swine, and non-human primates; however, infections in humans have also been reported [reviewed in (Carocci and Bakkali-Kassimi, 2012)]. The wide range of animals susceptible to infection makes EMCV a potential zoonotic agent. Additionally, cultured human cells that express the EMCV receptor, sialoglycoprotein vascular cell adhesion molecule 1 (VCAM-1), are susceptible to EMCV infection (Huber, 1994). EMCV infections in swine and rodents can lead to myocarditis, encephalitis, paralysis, type I diabetes, or even mortality [reviewed in (Carocci and Bakkali-Kassimi, 2012)]. No vaccine or antiviral therapeutic against EMCV infection is currently available. Since cardioviruses have a wide host range and the ability to cause multiple diseases, it is important to further understand the viral mechanisms employed by cardioviruses for development of antiviral therapeutics.

EMCV, like members of the enterovirus genus of picornaviruses, has a small, positive-sense RNA genome (~7.8 kb). Although members of the picornavirus family share many similarities in their genomic RNAs, the cardiovirus genome has distinct features that lead to differences in viral replication and host modulation mechanisms. Although it is covalently linked to the small viral protein, VPg (viral protein genome-linked), via a phosphotyrosyl linkage, the 5′ noncoding region (NCR) of EMCV differs from that of enteroviruses (i.e., coxsackievirus, poliovirus, human rhinovirus) in that it possesses a poly(C) tract, followed by pseudoknots with unknown functions, and a type II internal ribosome entry site (IRES) [reviewed in (Wimmer et al., 1993)]. The approximately 430-nucleotide type II IRES is more highly structured than the type I IRES that enteroviruses possess and is subdivided into five domains known as H-L. The IRES mediates initiation of cap-independent translation of the viral polyprotein, which includes a protein not expressed by enteroviruses. This EMCV-specific protein is the leader (L) protein, which is phosphorylated during infection and contains an N-terminal zinc finger domain (Cornilescu et al., 2008; Dvorak et al., 2001). The L protein, despite having no enzymatic activity, disrupts nucleo-cytoplasmic trafficking by indirectly phosphorylating the nucleoporin proteins and binding to Ran GTPase (Porter et al., 2006; Porter and Palmenberg, 2009). This is different from enterovirus-mediated disruption of nucleo-cytoplasmic trafficking, which is carried out by their viral proteinases 2A (Castello et al., 2011; Park et al., 2010) and 3C/3CD (Ghildyal et al., 2009); for review, see (Flather and Semler, 2015). Another major difference between EMCV and the enteroviruses is that unlike enterovirus 2A proteinases, the EMCV 2A protein has no proteolytic activity. In contrast to enterovirus 2A cleavage of eIF4G to shut off host cell translation, EMCV 2A expression triggers hypo-phosphorylation of the translation initiation factor 4E-BP1, leading to

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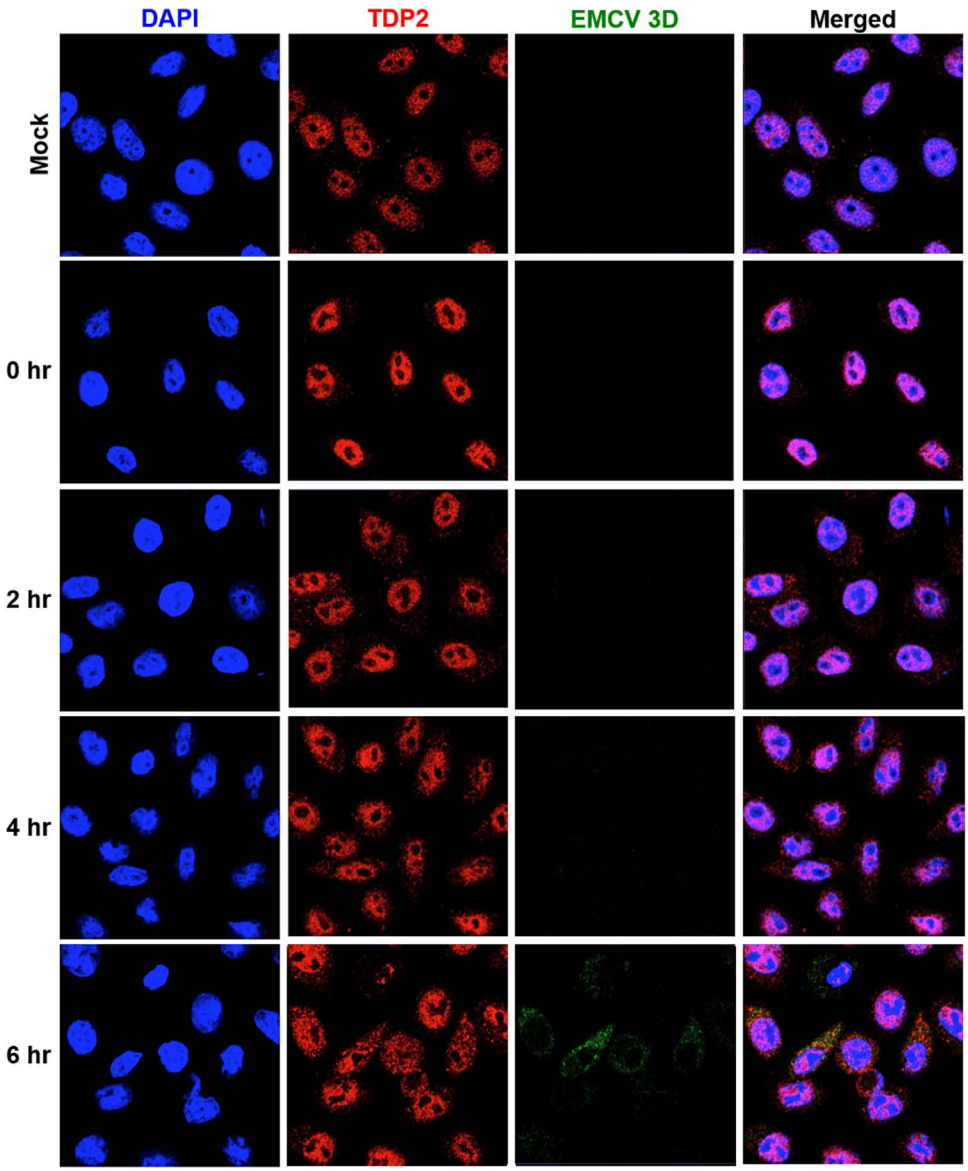

Fig. 1. TDP2 is re-localized from the nucleus to the cytoplasm during EMCV infection. HeLa cells were seeded on coverslips and either mock- or EMCV-infected at an MOI of 20. Cells were then fixed with formaldehyde at 0, 2, 4, or 6 h post-infection. Proteins were visualized by confocal microscopy using antibodies against human TDP2 (red) or EMCV 3D (green). Nuclei were counterstained with DAPI (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

inhibition of cap-dependent translation, albeit at a much slower rate than during poliovirus infection (Etchison et al., 1982; Jen et al., 1980; Svitkin et al., 1998). EMCV 2A was also shown to inhibit apoptosis (Carocci et al., 2011). Despite these mechanistic differences, EMCV, like enteroviruses, effectively alters the cellular environment to promote viral replication.

EMCV uses an additional strategy to enhance its replication cycle by inhibiting the host antiviral response. Previous studies showed that EMCV L protein interferes with IRF-3 dimerization, a step necessary for transcription of the interferon-alpha/beta genes (Hato et al., 2007). Additionally, EMCV, like poliovirus, targets the viral RNA sensor, RIG-I (Barral et al., 2009; Papon et al., 2009). It is important to note that not all host proteins function similarly during enterovirus and cardiovirus infections. For example, the cellular mRNA decay factor AUF1 acts as a restriction factor during coxsackievirus, poliovirus, or human rhinovirus infection, but during EMCV infection, levels of virus restriction appear to be host-cell dependent (Cathcart et al., 2013; Cathcart and Semler, 2014; Rozovics et al., 2012; Wong et al., 2013). In addition, host protein poly(rC) binding protein 2 (PCBP2) is required as an IRES trans-acting factor for enterovirus translation but not for EMCV translation (Walter et al., 1999). These previous findings illustrate some of the different pathways that are used by cardioviruses (compared to enteroviruses) to carry out viral gene expression and alter the cellular environment during infection.

In this report, we focused on the role of host protein 5′ tyrosyl-DNA phosphodiesterase 2 (TDP2) during EMCV infection. TDP2 possesses the VPg unlinkase activity that hydrolyzes the covalent phosphotyrosyl bond between VPg and the 5′ end of enterovirus RNAs (Virgen-Slane et al., 2012). TDP2 was also shown to be necessary for efficient enterovirus replication in murine cells (Maciejewski et al., 2015). Although it has not been confirmed that TDP2 functions as VPg unlinkase during cardiovirus infections, previous studies demonstrated that a cellular activity from mouse ascites Krebs II cells can hydrolyze the VPg-RNA linkage found in both poliovirus and EMCV RNA (Drygin et al., 1988; Drygin and Siianova, 1986). This activity was predicted to be a phosphodiesterase involved in repair of RNA and topoisomerase complexes (Gulevich et al., 2001, 2002). These characteristics resemble those of TDP2, which primarily removes tyrosine adducts resulting from topoisomerase II-mediated double-stranded DNA breaks (Cortes Ledesma et al., 2009). In this study, we sought to determine if TDP2 plays a role during EMCV infection and if its activity is modified by viral functions. Our previous work found that TDP2 was divergently required for enterovirus infections, with coxsackievirus B3 replication showing the highest levels of dependence on TDP2 (Maciejewski et al.,